Resistance to Leaf Scald Disease Is Associated with Limited Colonization of Sugarcane and Wild Relatives by *Xanthomonas albilineans*

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Accepted for publication 22 August 1997.

ABSTRACT

Rott, P., Mohamed, I. S., Klett, P., Soupa, D., de Saint-Albin, A., Feldmann, P., and Letourmy, P. 1997. Resistance to leaf scald disease is associated with limited colonization of sugarcane and wild relatives by *Xanthomonas albilineans*. *Phytopathology* 87:1202-1213.

A streptomycin- and rifampicin-resistant mutant of *Xanthomonas albilineans* was used to study symptom expression of leaf scald disease (LSD) and colonization of sugarcane (*Saccharum* spp.) and its wild relatives by this bacterial pathogen. A total of 40 sugarcane cultivars and 15 clones from the *Saccharum* complex that differed in resistance to LSD were inoculated by a decapitation technique in both field and greenhouse experiments. In the plant crop, disease severity varied between 0 for the most resistant genotypes and 100 for the most susceptible ones. Resistance to LSD was characterized by limited colonization of the host plant by *X. albilineans*. Although almost all genotypes were colonized by the

pathogen, the greatest bacterial population densities were found in the susceptible cultivars. There was a high correlation between disease severity and pathogen population in the apex. Several genotypes exhibited no or slight symptoms even though they were highly colonized in the upper and/or basal nodes of stalks. Two mechanisms, therefore, may play an important role in resistance to LSD: resistance to colonization of the apex, which is characterized by absence of symptoms, and resistance to colonization of the upper and lower parts of the stalk. In contrast, disease severity and pathogen population densities in the first ratoon crop in the field were nil or very low in the stalks, except for the highly susceptible cv. CP68-1026. Sugarcane ratoons, therefore, may recover from the disease after plant cane infection. Nevertheless, because low levels of the pathogen were still detected in some stalks, it is possible that LSD could develop from latent infections if favorable environmental conditions occur.

Leaf scald, a vascular disease caused by *Xanthomonas albilineans*, is a major disease of sugarcane (31). It can cause severe yield losses and is economically important in many locations (25, 31,46). In Guadeloupe, leaf scald disease (LSD) causes large reductions in yield (42) and sugar content and affects juice purity in susceptible cultivars (P. Rott, *unpublished results*). LSD occurs in most sugarcane-producing countries of the world, and recent outbreaks have occurred in several areas, including the Dominican Republic, Florida, Guadeloupe, Louisiana, Mexico, and Mauritius. The reasons for these outbreaks are unknown, but the introduction of a new strain of *X. albilineans* is suspected in Florida (12).

Several symptoms are expressed in LSD, varying in severity from a single, white, narrow, sharply defined leaf stripe to death of shoots or entire plants. A common symptom in mature diseased cane is the abnormal formation of side shoots on stalks, and basal side shoots tend to develop more rapidly than those higher up, whereas the opposite occurs in healthy stalks. One of the troublesome aspects of the disease is that many sugarcane cultivars can tolerate the pathogen without exhibiting symptoms or the symptoms escape detection (19,31,38). Planting healthy seed cane and using resistant cultivars are the most efficient means of control (15,31,41,51). Screening trials to evaluate resistance are carried out in many countries where the disease is a problem, but assessment of cultivar reactions is difficult and time-consuming. Assessments generally are based on observation of symptoms after ar-

tificial inoculation (22,29,50). Because symptoms do not always develop, this approach has been abandoned by many sugar industries (51).

Screening sugarcane for resistance on the basis of pathogen population densities recently has been used successfully for ratoon stunting disease (RSD), a vascular bacterial disease of sugarcane caused by *Clavibacter xyli* subsp. *xyli* (10,27,32,33). This method also might be applied to LSD; however, very little information is currently available about the growth of *X. albilineans* in sugarcane tissues. Infectivity titration appears to be promising for assessing resistance to leaf scald among sugarcane cultivars (24). Recently, we showed that high populations of *X. albilineans* are necessary but not sufficient for development of disease symptoms (37). In this study, it appeared that colonization of sugarcane was inversely correlated with resistance to LSD, but only a few cultivars were tested.

The purpose of our study was to investigate the relationship of stalk colonization to resistance of sugarcane and its wild relatives to LSD. A total of 40 sugarcane cultivars (interspecific hybrids of *Saccharum*) and 15 clones of the *Saccharum* complex that differ in resistance to the disease were inoculated by a decapitation technique in field and greenhouse experiments. Disease severity and pathogen populations at different locations in the stalk were examined at different stages of plant development.

MATERIALS AND METHODS

Bacterial strain and inoculation technique. *X. albilineans* strain GPE5SR (37), which carries spontaneous mutations for resistance to streptomycin (50 µg/ml) and rifampicin (50 µg/ml), was used to

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Publication no. P-1997-1001-01R

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inoculate sugarcane. This mutant is similar to its parent in pathogenicity (37). Inoculation was done by a decapitation technique described by Koike (21), but the stalks were not covered with an aluminum cap. In the field and greenhouse, the stalks were cut above the growing point (third dewlap [outer region of the leaf joint at the junction of the leaf sheath and leaf lamina] from the top) with pruning shears that had been dipped in a bacterial suspension (10^8 CFU/ml). In the field, inoculum was sprayed on the cut surface with a hand-held sprayer; in the greenhouse, 500 μ l of inoculum was deposited on the cut surface with a pipette. Control plants were treated in the same manner with distilled water and included in all experiments as procedural checks, i.e., to detect natural infections and show the mechanical effects of the inoculation procedure.

At the end of the field experiments, plants showing disease symptoms were tested to determine whether they contained strain GPE5SR or a wild-type strain of *X. albilineans*. Leaf tissue was cut into small fragments in petri plates containing sterile distilled water, and after 1 h, the suspension was plated on two selective media. The first medium was XAS, which was developed by Davis et al. (11) for detection of wild-type strains of *X. albilineans*, supplemented with propiconazole (10 μ g/ml). The second medium was mutant selective (MS), which consisted of Wilbrink's modified medium (39) supplemented with cycloheximide (50 μ g/ml), benomyl (12.5 μ g/ml), propiconazole (10 μ g/ml), streptomycin (50 μ g/ml), and rifampicin (50 μ g/ml). Only plants containing isolates able to grow on MS medium were considered infected by strain GPE5SR. Visual identification of *X. albilineans* colonies was verified randomly by serology (40).

Plant material and experimental design. *Field experiments.* Two separate field trials were set up in Guadeloupe. Twelve sugarcane cultivars with different resistance levels to LSD were used in the first experiment: B64277, B69379, B69566, B8008, Co1148, Co6415, CP68-1026, CP68-1067, M31/45, M147/44, Q63, and R570 (Table 1). Single-bud, i.e., one-node, cuttings of each cultivar were soaked for 48 h in water at ambient temperatures and treated with hot water at 50°C for 3 h to eliminate natural infections by *X. albilineans* (47). The cuttings were planted in the greenhouse. The resulting plants were grown for 4 months prior to transplanting to the field during February 1991 (8 to 26 plants per cultivar), one plot per cultivar. Eight-month-old plants were divided into two equal groups, and their stalks were inoculated with either the pathogen or water (control). All primary shoots and tillers were inoculated (≈ 10 nodes per stalk).

In the second experiment, disease-free tissue-cultured plants of four cultivars differing in resistance to LSD were planted in the field: B8008, R570, B69566, and CP68-1026 (Table 1). Tissue-cultured plantlets were propagated in vitro and transferred to the greenhouse as described by Feldmann et al. (16) and Paulet and Glaszmann (28). They were grown for 4 weeks in the greenhouse and transplanted to the field during July 1991, using a randomized complete block design with eight replications. In this design, a replication was equivalent to a block. Each plot contained two 6-m rows (9 plants and 70 to 100 tillers per row) per treatment. Each treatment consisted of either inoculated or control plants of a single cultivar. Five-month-old plants were inoculated with either the pathogen or distilled water (control). All primary shoots and tillers were inoculated (approximately three to four nodes per stalk). Within each plot, one row was used to monitor pathogen populations, and the other row was used to assess disease. Plants were grown according to normal commercial practices (14).

Greenhouse experiments. Three separate greenhouse experiments were set up in Montpellier, France. The first experiment included six sugarcane cultivars with different levels of resistance to LSD: B69379, B69566, B8008, Co6415, CP68-1026, and R570 (Table 1). All plants were derived from disease-free tissue-cultured plantlets and transferred to the greenhouse as described by Paulet and Glaszmann (28). The second experiment included 28 sugarcane

cultivars with known or unknown resistance levels to LSD (Table 1): B70462, Co740, Co6304, H70-0144, IAC48-65, IAC58-480, L66-97, Mana, M2173/63, My53-53, My54-62, N55/805, Ni1, Phil62-120, PR1016, Q84, Q90, Q110, Q115, Q117, Q120, Q124, R573, RB705375, RB735275, SP70-1078, SP70-1423, and Triton. The second greenhouse experiment was established with single-node cuttings from the stalks of disease-free plants. These plants were derived from tissue-cultured plantlets grown in a quarantine greenhouse (3). The third experiment included 15 clones from the *Saccharum* complex (Table 2) that were derived from single-node cuttings from a germ plasm collection at Montpellier.

TABLE 1. Characteristics of the 40 sugarcane cultivars used in this study

Cultivar	Origin	Resistance level to LSD ^a	Location	Reference
B64277	Barbados	S	Guadeloupe	Authors ^b
			Martinique	Authors
B69379	Barbados	S	Guadeloupe	42
B69566	Barbados	S	Guadeloupe	42
			St. Kitts	Authors
B70462	Barbados	...	...	...
B8008	Barbados	R	Guadeloupe	42
Co740	Coimbatore, India	VR	Australia	43
Co1148	Coimbatore, India	VR	Australia	43
		R	Guadeloupe	Authors
		S	India	52
		S	Sumatra	36
Co6304	Coimbatore, India	...	...	...
Co6415	Coimbatore, India	R	Guadeloupe	42
CP68-1026	Canal Point, U.S.	VS	Ivory Coast	6
			Guadeloupe	42
CP68-1067	Canal Point, U.S.	I	Guadeloupe	Authors
		VS	Ivory Coast	6
H70-0144	Hawaii	R	Hawaii	36
IAC48-65	Brazil	R	Brazil	35
IAC58-480	Brazil	...	...	...
L66-97	Louisiana, U.S.	...	...	...
Mana	Fiji	...	...	...
M31/45	Mauritius	R	Mauritius	26
		S	South Africa	36
M2173/63	Mauritius	...	...	...
M147/44	Mauritius	S	Madagascar	35
		S	Mozambique	35
		VS	Mauritius	26
My53-53	Mayari, Cuba	...	...	...
My54-62	Mayari, Cuba	...	...	...
N55/805	Natal, S. Afr. ^c	R	South Africa	34
		VR	Australia	43
Ni1	Okinawa, Japan	...	...	...
Phil62-120	Philippines	...	...	...
PR1016	Puerto Rico	...	...	...
Q63	Queensland, Aust. ^d	S	Numerous	34
		VS	Ivory Coast	6
Q84	Queensland, Aust.	VR	Australia	35
Q90	Queensland, Aust.	VR	Australia	34
Q110	Queensland, Aust.	VR	Australia	34
Q115	Queensland, Aust.	VR	Australia	34
Q117	Queensland, Aust.	VR	Australia	34
Q120	Queensland, Aust.	VR	Australia	34
Q124	Queensland, Aust.	R	Australia	34
R570	Réunion, France	R	Réunion	29
		I	Guadeloupe	42
		S	Mauritius	26
R573	Réunion, France	...	...	...
RB705375	Brazil	...	...	...
RB735275	Brazil	I	Brazil	34
SP70-1078	São Paulo, Brazil	R	Brazil	34
SP70-1423	São Paulo, Brazil	...	...	...
Triton	CSR, Aust.	VR	Australia	43

^a VR = very resistant, R = resistant, I = intermediate, S = susceptible, and VS = very susceptible.

^b Unpublished observations of P. Rott, I. S. Mohamed, P. Klett, D. Soupa, A. de Saint-Albin, P. Feldmann, and P. Letourmy.

^c South Africa.

^d Australia.

In all three experiments, each cutting or tissue-culture derived plantlet was individually planted in 6-liter pots containing a mixture of peat moss and volcanic rock (1:1, vol/vol). The pots used in the first experiment (six cultivars) were arranged in the greenhouse according to a randomized block design with four replications. In each block, each experimental unit contained six pots of a single cultivar consisting of either inoculated (four pots) or control plants (two pots). The pots used in the second experiment (28 cultivars) were distributed in the greenhouse according to a totally randomized design with five replications per cultivar. Each experimental unit consisted of one inoculated plant and one control plant per cultivar. The pots used in the third experiment (15 clones) were distributed in the greenhouse according to a randomized block design with three replications. Each experimental unit contained four pots of a single treatment consisting of either inoculated or control plants of a single clone. All primary stalks (approximately four to five nodes) of 5-month-old plants were inoculated with either the pathogen or distilled water (control).

Disease assessment. Symptoms were recorded monthly in the field and greenhouse. Disease severity (varying between 0 for the most resistant genotypes and 100 for the most susceptible ones) was rated by the procedure described by Rott et al. (42). Briefly, all inoculated stalks were rated individually, using a symptom severity scale ranging from 0 to 5. Ratings were used to calculate mean disease severity (DS): $DS = [(1 \times FL + 2 \times ML + 3 \times CB + 4 \times N + 5 \times D) / 5 \times T] \times 100$, where FL = number of stalks with one or two pencil-line streaks (score 1), ML = number of stalks with more than two pencil-line streaks (score 2), CB = number of stalks with

leaf chlorosis or bleaching (score 3), N = number of stalks with leaf necrosis (score 4), D = number of dead stalks or stalks with side shooting (score 5), and T = total number of stalks. The same rating system was used for disease severity in leaves 1 month after inoculation and in noninoculated leaves at all other time points. However, a score of 5 was attributed to stalks with dead inoculated leaves 1 month after inoculation.

Sampling procedure. To use a common sampling procedure for both field experiments, 25 stalks were sampled randomly per cultivar at each time point among those inoculated with the pathogen. The samples were taken more or less equally from different stools of the single plot in the first experiment and from across the eight replications in the second experiment (3 or 4 stalks sampled of 70 to 100 stalks per cultivar per replication). The experimental design (eight blocks) of the second experiment was used to obtain preliminary data on the correlation between yield reduction and pathogen population densities in stalks (data not shown). The 25 stalks per cultivar were sampled randomly 3 (experiment 1) or 7 (experiment 2) months after inoculation of plant cane. The same procedure was used when sugarcane was 3 and 12 (experiment 1) or 9 (experiment 2) months old in the first ratoon crop. The 25 stalks were divided and pooled at random into 5 groups of 5 stalks each. The symptoms were recorded for each stalk before removal of the different parts to be examined for bacterial colonization: the apex ($\approx 1 \times 2$ cm of tissue containing the apical meristem) and a 5-cm single-node section in the upper third and a 5-cm single-node section in the lower third of each stalk. The stalk sections were peeled with a potato peeler to reveal only the inner vascular tissue. An

Fig. 1. Disease progress in stalks in the plant cane and first ratoon crops of 12 sugarcane cultivars inoculated with *Xanthomonas albilineans* GPE5SR (field experiment 1). Plant cane was 8 months old at time of inoculation. H = harvest of the plant crop. Values are the means of all stalks of 4 to 13 stools per cultivar (one plot).

internode section also was taken from different locations in each stalk to determine whether it was infected (described below).

In greenhouse experiments, one or two inoculated stalks were collected 3 months after inoculation from each block or replication. The symptoms were recorded for each stalk before removal of the different parts to be examined for bacterial colonization: the apex ($\approx 0.5 \times 1$ cm) and a 4-cm single-node section in the upper third and a 4-cm single-node section in the lower third of each stalk. The node section was peeled as described for field experiments.

Measurement of bacterial population densities. In field experiments, each pooled sample of five apices or five node sections was weighed and homogenized in 150 (10- to 15-g apex) to 400 ml (100- to 200-g node section) of TBS buffer (2.42 g of Tris, 8 g of NaCl, 1,000 ml of distilled water, pH 7.5) with a blender, 2 times for 30 s each (first on low speed and then on high speed). The blended sugarcane extracts were kept at room temperature without agitation for 2 h and filtered through a cloth. In plant cane, serial dilutions of the filtrates were plated in triplicate on MS medium containing streptomycin and rifampicin. In the first ratoon crop, the filtrates were streaked on the MS medium with a Spiral plater system (Interscience, Saint-Nom La Bretèche, France) (20). Bacterial colonies were counted after a 7-day incubation at 28°C. Visual identification of *X. albilineans* colonies was verified randomly by serology (40). In greenhouse experiments, samples were treated in the same manner, but each apex or node section was individually weighed and homogenized. Sugarcane tissues were homogenized in 150 (1.5- to 4-g apex) to 200 ml (5- to 20-g node section) of TBS buffer.

Determination of infected stalks. Different techniques were used according to the experiment and age of the sugarcane. The "stalk blot isolation" technique (11) was used for all stalks sampled in the first field experiment. A freshly cut surface of an internode section was pressed in duplicate onto MS medium containing streptomycin and rifampicin. An internode in the middle of each stalk was sampled in plant cane, and an internode in the lower third of each stalk was sampled in the first ratoon crop. Growth of *X. albilineans* was recorded after 7 days at 28°C. In the second field experiment, three isolation methods were used to determine the percentage of colonized stalks at different ages of sugarcane. The first method, stalk blot isolation, was applied to the plant cane as described above, but an internode section was sampled from both the basal and upper parts of each stalk. In the second method, leaf tissue of sugarcane shoots was sampled during the first 3 months of the first ratoon crop. The base (2 to 3 cm) of shoots was cut into small fragments in petri plates containing sterile distilled water, and after 1 h, the suspension was plated on MS medium. In the third method, sugarcane sap was extracted at 4 months or later into the first ratoon crop by squeezing an internode section with

pliers. Extracts were collected in tubes, and 50- μ l aliquots were streaked on the selective medium. Visual identification of *X. albilineans* colonies was verified randomly by serology (40).

Statistical analysis. SAS computer programs (SAS Institute Inc., Cary, NC) were used for data analysis. The area under the disease progress curve for each replication of each cultivar was calculated with the data from all observation times in plant crop, and the cultivars were compared by analysis of variance (4). The relationship between disease severity and pathogen population density was investigated by regular regression analysis after log-transformation of population density data, i.e., $\log[(\text{CFU/g fresh tissue}) + 1]$. Two models were investigated: simple linear and quadratic regressions.

RESULTS

Disease progress during the first two crop cycles in the field.

In the first field experiment, 1 month after inoculation of the 8-month-old plants disease severity in inoculated leaves was high for all 12 cultivars ($DS \approx 80$). Greater variation in disease severity was detected among cultivars after 2 months, when observations were made on newly developed leaves rather than on inoculated leaves, which had become senescent. In plant cane, different patterns of disease progress were observed, and four groups were distinguished (Fig. 1): highly susceptible (cvs. CP68-1026, Q63, and B64277), for which disease severity increased rapidly and reached values >95 ; susceptible (cvs. B69566, B69379, and M147/44), for which disease severity was >60 but did not reach maximum values; intermediate (cvs. CP68-1067, M31/45, and R570), for which disease severity was >20 but ≤ 60 ; and resistant (cvs. B8008, Co1148, and Co6415), for which disease severity decreased rapidly and was ≤ 20 at 4 months after inoculation. Groupings could not be distinguished in the first ratoon crop (Fig. 1), in which, with the exception of highly susceptible cv. CP68-1026, little disease developed. In cv. CP68-1026, disease severity was maximal ($DS = 43$) in mature cane, i.e., when stalks were 11 to 12 months old. The disease severity of the other 11 cultivars was low or nil. Leaf scald symptoms were observed on some young tillers that appeared during the season and on a few mature stalks with side shoots (B69566, CP68-1067, and Q63). However, these mature stalks, as well as a few control stalks, had succumbed to natural infection by a wild-type strain of *X. albilineans* that was susceptible to streptomycin and rifampicin.

Fig. 2. Disease progress in stalks in the plant cane and first ratoon crops of four sugarcane cultivars inoculated with *Xanthomonas albilineans* GPE5SR (field experiment 2). Plant cane was 5 months old at time of inoculation. H = harvest of the plant crop. Values are the means of eight replications of all stalks from one 6-m sugarcane row. The *F* test for area under the disease progress curve for cultivar effect was significant at $P = 0.0001$. Each cultivar differed from the other according to mean separation based on least significant difference ($P = 0.05$).

TABLE 2. Clones of the *Saccharum* complex used in this study

Clone	Species ^a	Origin
CHUNNEE	<i>Saccharum barberi</i> (p)	India
COIMBATORE	<i>S. spontaneum</i> (w)	India
GLAGAH1286	<i>S. spontaneum</i> (w)	Indonesia
GLAGAH WT	<i>S. spontaneum</i> (w)	Indonesia
IJ76-176	<i>Erianthus arundinaceus</i> (w)	Indonesia
MANDALAY	<i>S. spontaneum</i> (w)	Burma
MOENTAI	<i>S. spontaneum</i> (w)	India ?
NG28-289	<i>S. robustum</i> (w)	Papua New Guinea
NG28-7	<i>E. arundinaceus</i> (w)	Papua New Guinea
NG57-30	<i>S. officinarum</i> (p)	Papua New Guinea
PURI1557	<i>S. sinense</i> (p)	China
RAKHRA1323	<i>S. barberi</i> (p)	India
SARETHA	<i>S. barberi</i> (p)	India
SES49	<i>S. spontaneum</i> (w)	India
X	<i>E. arundinaceus</i> (w)	Indonesia

^a In parentheses, p = primitive sugarcane cultivar and w = wild relative of sugarcane.

Similar patterns of disease progress were observed in the second field experiment (Fig. 2). At 1 month after inoculation of the 5-month-old plants, disease severity in inoculated leaves was high for all four cultivars ($DS \approx 80$) but varied among the cultivars 2 months after inoculation. Disease severity decreased rapidly for resistant cv. B8008 and was below 10 for the rest of the experiment. Disease severity increased, however, for highly susceptible cv. CP68-1026 and at 3 to 5 months after inoculation remained higher than 90. Disease progress in susceptible cv. B69566 and intermediate cv. R570 was similar to the progress in CP68-1026 but at lower levels. Disease severity decreased in all sugarcane cultivars between 5 and 7 months after inoculation (10- to 12-month-old plants). This decline was most obvious in cv. R570, mainly because of the absence of symptoms in leaves that developed several weeks after inoculation. In the case of cvs. B69566 and CP68-1026, the slight decrease was most likely due to noninoculated tillers that developed after inoculation and could not be distinguished from the inoculated stalks at the time of disease assessment. As in the first field experiment, the various cultivars could not be differentiated on the basis of disease severity in the first ratoon crop, because disease did not develop (Fig. 2). Disease severity was low even in highly susceptible cv. CP68-1026 ($DS \leq 14$). About 10% of the control stalks of this cultivar also exhibited LSD symptoms at the end of the plant and ratoon crops but were not infected with strain GPE5SR. The pathogen isolated from more than 50 diseased control samples was susceptible to streptomycin and rifampicin.

Disease severity and pathogen population densities in the field.

At 3 months after inoculation in field experiment 1, disease severity varied in the sampled stalks between 0 for Co6415, the most resistant cultivar, and 99.5 for CP68-1026, the most susceptible cultivar (Fig. 3). All sugarcane cultivars were colonized by *X. albilineans*. The percentage of infected stalks reached 100% for the susceptible cultivars and was not less than 76% for the resistant ones. Mean pathogen population densities in stalks within cultivars varied between 30 and 5.2×10^8 CFU/g of fresh tissue in the node sections and between undetectable levels and 8.7×10^8 CFU/g of fresh tissue in the apex. More bacteria were detected in the upper than in the lower parts of stalks in most cultivars. Highly significant ($P < 0.0001$) linear regressions were obtained when the population density estimates (log base-10 transformation) for pooled samples from nodal or apical tissues were regressed on the mean disease severity of the same samples (Fig. 4).

In contrast, in the first ratoon crop, disease severity in 3- and 12-month-old stalks was nil, except for cv. CP68-1026. Of 25 stalks of this highly susceptible cultivar, 20 (80%) were heavily infected with *X. albilineans* at the end of the crop, and disease severity was 56. The mean pathogen populations increased in the lower node section of cv. CP68-1026 from 3.2×10^6 CFU/g of fresh tissue in 3-month-old stalks to 1.3×10^8 CFU/g of fresh tissue in 12-month-old stalks. Populations in the apices were 4.0×10^4 CFU/g of fresh tissue at 3 months and reached 2.5×10^8 CFU/g of fresh tissue at 12 months. The population densities at harvest (12 months old) in the first ratoon crop were equivalent to those observed at harvest

Fig. 3. Disease severity and bacterial populations 3 months after inoculation of 12 sugarcane cultivars in the plant crop with *Xanthomonas albilineans* GPE5SR (field experiment 1). Values are the means ($\pm$ standard error) of five replications of the pooled samples for each tissue.

in plant cane 4 months after inoculation. The pathogen rarely was detected in stalks of the other 11 cultivars during the first ratoon crop when the presence of the bacteria was tested by blotting an internode section on MS medium. However, the pathogen was detected in several stalks when the tissues were homogenized and extracts were quantitatively streaked on selective medium with a Spiral plater. The number of infected stalks could not be determined accurately by this technique because the samples were pooled. However, the mean bacterial populations could be evaluated, because the minimum detection levels of this technique were very low, i.e., generally between 10 and 20 CFU/g of fresh tissue for the node sections and between 50 and 100 CFU/g of fresh tissue for the apices. Mean populations of the pathogen were very low in each of the 11 sugarcane cultivars for both sampling dates in the first ratoon crop and for each part of the stalk that was studied. The populations ranged from undetectable to 6.3×10^3 CFU/g of fresh tissue.

Although various patterns of disease progress were observed in plant cane of the second field experiment, nearly all stalks of cvs.

R570, B69566, and CP68-1026 were colonized by the pathogen (Table 3). The percentage of colonized stalks for the resistant cv. B8008 were lower but nevertheless relatively high (60 to 85%). At 7 months after inoculation, disease severity varied in the sampled stalks between 0 for the resistant cv. B8008 and 100 for the highly susceptible cv. CP68-1026 (Fig. 5). Pathogen population densities in the stalks varied between 2.5×10^2 and 1.0×10^9 CFU/g of fresh tissue in the upper part and between 5.0×10^6 and 6.3×10^8 CFU/g of fresh tissue in the lower part. Unlike what we observed in the first field experiment, the populations of *X. albilineans* appeared to be similar in both parts of the stalk, with the exception of cv. B8008. In this cultivar, pathogen populations were 10^4 times higher in the basal part than in the upper part. The relationship between disease severity and pathogen population density was not investigated by regular regression analysis because only four data points were available per location. However, disease severity was not always correlated to the amount of bacteria in the nodal sections of the stalk. Indeed, disease severities of the intermediate (R570) and susceptible (B69566) cultivars were different, but the corresponding pathogen populations were similarly high in the upper nodal section (Fig. 5). Pathogen populations in the lower nodal section also were high in the resistant (B8008) and susceptible (B69566) cultivars, whereas the corresponding disease severity was clearly different (0 versus 84).

In the first ratoon crop, only a few stalks were colonized by *X. albilineans*, and the highest values were observed after 3 months of growth (Table 3). The pathogen was detected regardless of age in sugarcane cv. CP68-1026 (18 to 98% of colonized stalks) but never in cv. R570. It was found only during the first months of growth in the two remaining cultivars. Disease severity of the stalks sampled after 9 months in the first ratoon crop was nil, except for highly susceptible cv. CP68-1026. Although disease severity was low ($DS = 12$), cv. CP68-1026 was colonized by the pathogen, but the population densities were lower than those observed in plant cane: 5.0×10^4 CFU/g of fresh tissue in the upper node section and 6.3×10^6 CFU/g of fresh tissue in the lower node section. The pathogen was either not detected or was detected at very low levels in the three remaining cultivars.

Disease severity and pathogen population densities in the greenhouse. At 3 months after inoculation of the six sugarcane cultivars in the first greenhouse experiment, disease severity varied in the sampled stalks between 2.5 for cv. Co6415 and 75 for cv. B69379

Fig. 4. Relationship between mean disease severity and mean population densities (log base-10 transformation) of *Xanthomonas albilineans* in three parts of 12 sugarcane cultivars with leaf scald disease (field experiment 1, 3 months after inoculation in the plant crop).

TABLE 3. Percentage of colonized plant and ratoon cane stalks in four sugarcane cultivars inoculated with *Xanthomonas albilineans* GPE5SR (field experiment 2, plant cane 5 months old at time of inoculation)

Sugarcane age (month)	Percentage of colonized stalks ^a in each cultivar			
	B8008 (R) ^b	R570 (Unknown)	B69566 (S)	CP68-1026 (VS)
Plant cane				
8	80	100	100	100
9	60	95	100	100
10	65	90	90	100
12	85	90	90	100
First ratoon crop				
1	2	0	5	40
2	0	0	0	38
3	22	0	30	98
4	0	0	0	32
6	0	0	0	32
8	0	0	0	18
9	0	0	0	52

^a Determined by stalk blot isolation in plant cane (20 basal and 20 upper internode sections per cultivar), maceration of leaf tissue during the first 3 months of the first ratoon crop (40 shoots per clone), sap extraction after 4 months in the first ratoon crop (40 basal internode sections per cultivar).

^b R = resistant, S = susceptible, VS = very susceptible. Reactions were determined previously (Table 1).

(Fig. 6). These six cultivars reacted similarly in greenhouse and field experiments, i.e., resistant cvs. Co6415 and B8008 exhibited few symptoms, intermediate cv. R570 showed an intermediate reaction, and susceptible or highly susceptible cvs. B69566, B69379, and CP68-1026 were heavily diseased. However, disease severity on the latter cultivars was lower in the greenhouse than in the field. All sugarcane cultivars were colonized by *X. albilineans*.

Mean pathogen population densities in stalks within cultivars varied between 3 and 5.0×10^7 CFU/g of fresh tissue in the apex and between 40 and 2.0×10^8 CFU/g of fresh tissue in the node sections.

Disease severity of the 28 sugarcane cultivars tested in the second greenhouse experiment varied in the sampled stalks 3 months after inoculation between 0 (14 cultivars) and 100 (cv. H70-0144) (Fig. 7). All cultivars, except Co6304, were colonized by *X. al-*

Fig. 5 Disease severity and bacterial populations 7 months after inoculation of four sugarcane cultivars in the plant crop with *Xanthomonas albilineans* GPE5SR (field experiment 2). Values are the means ($\pm$ standard error) of five replications of the pooled samples for each tissue.

Fig. 6 Disease severity and bacterial populations 3 months after inoculation of six sugarcane cultivars with *Xanthomonas albilineans* GPE5SR (greenhouse experiment 1). Values are the means ($\pm$ standard error) of eight replications, each containing a single stalk.

bilineans. Mean pathogen population densities in stalks within cultivars varied between undetectable levels and 2.5×10^{10} CFU/g of fresh tissue in the apex and between undetectable levels and 5.0×10^9 CFU/g of fresh tissue in the node sections. In the case of the 15 clones of the *Saccharum* complex 3 months after inoculation, disease severity varied in the sampled stalks between 0 and 90 (third greenhouse experiment, Fig. 8). All clones were colonized by *X. albilineans*. Mean pathogen population densities in stalks within cultivars varied between undetectable levels and 2.0×10^8 CFU/g of fresh tissue in the apex and between undetectable levels and 3.2×10^8 CFU/g of fresh tissue in the node sections.

A highly significant ($P < 0.0015$) correlation was obtained for all greenhouse experiments when the population density estimates (log base-10 transformation) for samples from apical tissues were linearly regressed on the mean disease severity of the same samples (Figs. 9 to 11). Some genotypes that exhibited no symptoms were not or only slightly colonized by the pathogen, irrespective of stalk location. In contrast, high populations of *X. albilineans* were found in the upper and/or lower stalk locations in several genotypes that showed different degrees of disease severity. In these genotypes, there was no linear relationship between disease severity and pathogen populations in nodal tissues, but pathogen populations reached a plateau (Figs. 9 to 11). In the basal nodes, a critical population

size was reached at low levels of disease, and it remained approximately the same regardless of disease severity.

DISCUSSION

Assessment of sugarcane resistance to LSD, so far, has been based solely on the severity or frequency of symptoms that developed naturally in fields or after artificial inoculation of plants in specific trials (22,29,31,50). In our studies, sugarcane cultivars known to be resistant (Co6415 and B8008) exhibited no or slight symptoms, whereas cultivars known to be susceptible (B69566, B69379, and CP68-1026) showed severe symptoms. Various degrees of disease severity were observed in other sugarcane cultivars or clones of the *Saccharum* complex. Although nearly all stalks of the 12 sugarcane cultivars inoculated in the field were infected, numerous stools apparently were able to recover from the infection between plant cane and first ratoon crops. This remission was not directly related to cultivar resistance, because most susceptible cultivars did not exhibit severe symptoms and were not colonized by *X. albilineans* GPE5SR in the first ratoon crops. Nevertheless, a question still exists as to whether the pathogen was present below our level of detection or in a nonculturable state. Other studies likewise have found that populations of *X. albilineans* can escape detection with other diagnostic techniques, such as sero-

Fig. 7. Disease severity and bacterial populations 3 months after inoculation of 28 sugarcane cultivars with *Xanthomonas albilineans* GPE5SR (greenhouse experiment 2). Values are the means ($\pm$ standard error) of five replications, each containing a single stalk. Cultivars: 1 = Co6304, 2 = Q120, 3 = Phil62-120, 4 = Q84, 5 = Q117, 6 = L66-97, 7 = N55/805, 8 = IAC48-65, 9 = Co740, 10 = Mana, 11 = Ni1, 12 = My53-53, 13 = Q115, 14 = Q90, 15 = RB735275, 16 = Triton, 17 = Q110, 18 = PR1016, 19 = Q124, 20 = B70462, 21 = M2173/63, 22 = My54-62, 23 = IAC58-480, 24 = SP70-1078, 25 = RB705375, 26 = R573, 27 = SP70-1423, and 28 = H70-0144.

logical methods (1,5,11,23). In our studies, bacterial population densities often were close to the minimum detection level when the pathogen was isolated in the first ratoon crop. Furthermore, the bacteria may have been present in parts of the plant that were not examined, such as the roots or stubble. One also could speculate that the remission of the disease in the ratoon crops, after expression of disease symptoms in the plant crops, was related to reduced virulence or fitness of the pathogen in the first ratoon crops, due to the antibiotic resistance markers present in the strain we used. This possibility cannot be ruled out, but similar remission also was observed in another field trial in which plants were inoculated with a wild-type strain of *X. albilineans* (the parental strain of the mutant used in these studies) (42). A similar phenomenon has been reported for other sugarcane diseases. For example, several susceptible cultivars show recovery in ratoon crops after plant cane infection by the sugarcane mosaic potyvirus (51). Similarly, Davis et al. (8) suggested that sugarcane becomes free of *C. xyli* subsp. *xyli* between the plant crop and first ratoon crop. In the case of LSD, an unknown mechanism of resistance may limit pathogen growth, or the bacteria may become dormant until environmental conditions are favorable for symptom expression.

Disease severity and pathogen population densities in the upper and lower parts of the stalk were correlated in the first field experiment, when the 12 sugarcane cultivars were sampled and rated 3 months after inoculation. However, this correlation was not found in the second field experiment or in the three greenhouse experiments, even when low and high populations densities were found

in several resistant and susceptible cultivars, respectively. Additionally, the highest populations generally were found in upper nodal sections in the first field experiment but not in the other experiments. The highest populations also were found in the upper part of the stalk by Comstock and Irey (5), who worked with naturally infected stalks. The implications of inoculating sugarcane by decapitation, in which infection begins at the top of the plant, cannot be overlooked in our studies. Several factors may be responsible for stalk colonization between various trials, such as the age and size of stalks at the time of inoculation, environmental conditions during the experiment, and time between inoculation and measurement of pathogen population densities. Results obtained in the second field experiment and in the three greenhouse experiments were similar. A major difference between the first field experiment and the other trials was the age and size of plants at the time of inoculation. Previous studies showed that pathogen populations in the basal portion of the stalk can remain stable for several months after reaching peak levels, even if disease severity diminishes (37). Therefore, it is likely that the populations of *X. albilineans* were measured in the first field experiment before peak levels were obtained, especially in the lower part of the stalk, which was the part furthest from the inoculation zone.

Pathogen populations in the apex were highly correlated with disease severity in all field and greenhouse experiments. Therefore, limited colonization of the apex can be considered a characteristic of resistance to LSD. In contrast, pathogen populations at the other stalk locations generally were not correlated with disease

Fig. 8. Disease severity and bacterial populations 3 months after inoculation of 15 clones of the *Saccharum* complex with *Xanthomonas albilineans* GPE5SR (greenhouse experiment 3). Values are the means ($\pm$ standard error) of six replications, each containing a single stalk.

severity, and several patterns of stalk colonization were observed after inoculation of the 40 sugarcane cultivars and 15 clones of the *Saccharum* complex. Susceptible plants that exhibited severe symptoms of LSD were always highly colonized in both the upper and lower parts of the stalk. However, resistant or intermediate plants, which exhibited no or only a few symptoms, were colonized differently by the pathogen: the stalks of some genotypes were not or only weakly colonized, whereas high populations were found in others. As a consequence, it appeared that colonization of the apex (correlated with symptom appearance) and colonization of the stalk are not always similar, and therefore, at least two different resistance mechanisms may be involved. These results agree with those of a previous study that indicated that high populations of *X. albilineans* are necessary but not sufficient for development of disease symptoms (37). The basis for the limited colonization of resistant plants by *X. albilineans* has not yet been elucidated. Similar studies of resistance to RSD suggested that the vascular anatomy of sugarcane may play an important role by restricting

the intraxylar spread of the pathogen in resistant plants (48,49). Differences in the degree of branching of the metaxylem and the number of xylem vessels that pass directly through stalk nodes without terminating may be important features that affect the spread of *X. albilineans* in stalks. If so, then RSD and LSD resistance could be related. However, this has not yet been shown. Furthermore, as with RSD of sugarcane, high populations of the pathogen at the bottom of the stalk may affect plant growth and yields even if no external symptoms are visible. Studies are in progress to examine this characteristic of LSD.

Resistance of plants to bacterial pathogens generally is associated with inhibition of multiplication or spread of the pathogen in infected tissues. Consequently, bacterial population densities generally are lower in resistant plants than in susceptible ones (2,8,9, 44,45,53). In the case of vascular pathogens, resistance appears to be correlated with restricted colonization of vascular tissues (7,13, 17,30). This already has been demonstrated for RSD of sugarcane caused by *C. xyli* subsp. *xyli* (8,9,18). Consequently, this charac-

Fig. 9. Relationship between mean disease severity and mean population densities (log base-10 transformation) of *Xanthomonas albilineans* in three parts of six sugarcane cultivars with leaf scald disease (greenhouse experiment 1, 3 months after inoculation).

Fig. 10. Relationship between mean disease severity and mean population densities (log base-10 transformation) of *Xanthomonas albilineans* in three parts of 28 sugarcane cultivars with leaf scald disease (greenhouse experiment 2, 3 months after inoculation).

teristic can be used in screening procedures for resistance to this disease (10,27,32,33). In our studies, resistance based on disease symptoms was not always correlated with the degree of colonization of the plant. Although the basis for cultivar differences in colonization is unknown, screening procedures for LSD resistance apparently can be based on pathogen population densities. This procedure appears to be all the more valuable because screening based on symptoms of LSD is not always efficient and can be impossible when symptoms do not develop. Ideally, one may want to analyze numerous stalks and cultivars in a commercial screening procedure. Because of technical constraints, particularly the time required to prepare and analyze the samples, only a few replications can be sampled in field experiments to determine pathogen population densities. Therefore, in our field study the samples were bulked, and tissues were not plated individually. As a consequence, the arithmetic mean for a population could be overestimated and the amount of resistance underestimated when only a few samples are colonized per pooled tissue. In such a sampling

procedure, therefore, it is important to determine the frequency of infection that can give additional information regarding resistance to LSD. The frequency of infection in plant crops in our study was generally very high (76 to 100%), and therefore, the arithmetic mean for a population can be considered representative for most cultivars. However, some highly infected plants may have biased some of the means. Because field and greenhouse experiments resulted in similar conclusions, the influence of biased data was considered minimal.

On the basis of this study, a cultivar should be considered resistant only if the basal part of the stalk is not extensively colonized. Cultivars that are colonized at the base of the stalk and do not exhibit symptoms should be considered "hazardous" until additional information regarding the movement of the pathogen in the plant becomes available. Because resistance based on colonization also was found in other clones of the *Saccharum* complex, wild relatives or primitive sugarcane cultivars may be useful in breeding programs as additional sources of resistance to LSD.

ACKNOWLEDGMENTS

This research was supported in part by the Conseil Régional de la Guadeloupe and by the Action Thématique Programmée CIRAD "Analyse des composantes de la résistance des plantes et application à l'amélioration des plantes." We thank R. Boisne-Noc, J. M'Passy, J. Chaume, M. Chatenet, M. J. Darroussat, M. Muller, J. F. Bousquet, and Y. Valmorin for technical assistance and C. Kaye for the critical review.

LITERATURE CITED

1. Autrey, L. J. C., Dookun, A., and Saumtally, S. 1989. Improved serological methods for diagnosis of the leaf scald bacterium *Xanthomonas albilineans*. Proc. Int. Soc. Sugar Cane Technol. 20:704-713.
2. Bailey, R. A. 1977. The systemic distribution and relative occurrence of bacteria in sugarcane varieties affected by ratoon stunting disease. Proc. S. Afr. Sugar Technol. Assoc. Annu. Congr. 51:55-56.
3. Baudin, P. 1984. Quarantaine de canne à sucre à Montpellier, France. Agron. Trop. 39:262-268.
4. Campbell, C. L., and Madden, L. V. 1990. Temporal analysis of epidemics. I: Description and comparison of disease progression curves. Pages 161-202 in: Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York.
5. Comstock, J. C., and Irey, M. S. 1992. Detection of the sugarcane leaf scald pathogen, *Xanthomonas albilineans*, using tissue blot immunoassay, ELISA, and isolation techniques. Plant Dis. 76:1033-1035.
6. Coulibaly, F., Baran, R., and Rott, P. 1988. La maladie de l'échaudure des feuilles en Côte d'Ivoire. Agron. Trop. 43:150.
7. Davis, M. J. 1989. Host colonization and pathogenesis in plant diseases caused by fastidious xylem-inhabiting bacteria. Pages 33-50 in: Vascular Wilt Diseases of Plants. E. C. Tjamos and C. Beckman, eds. NATO ASI Series, vol. H28. Springer-Verlag, Berlin.
8. Davis, M. J., Dean, J. L., and Harrison, N. A. 1988. Quantitative variability of *Clavibacter xyli* subsp. *xyli* populations in sugarcane cultivars differing in resistance to ratoon stunting disease. Phytopathology 78:462-468.
9. Davis, M. J., Dean, J. L., and Harrison, N. A. 1988. Distribution of *Clavibacter xyli* subsp. *xyli* in stalks of sugarcane cultivars differing in resistance to ratoon stunting disease. Plant Dis. 72:443-448.
10. Davis, M. J., Dean, J. L., Miller, J. D., and Shine, J. M., Jr. 1994. A method to screen for resistance to ratoon stunting disease of sugarcane. Sugar Cane 6:9-16.
11. Davis, M. J., Rott, P., Baudin, P., and Dean, J. L. 1994. Evaluation of selective media and immunoassays for detection of *Xanthomonas albilineans*, causal agent of sugarcane leaf scald disease. Plant Dis. 78:78-82.
12. Davis, M. J., Rott, P., Warmuth, C. J., Chatenet, M., and Baudin, P. 1997. Genetic variation within *Xanthomonas albilineans*, the sugarcane leaf scald pathogen. Phytopathology 87:316-324.
13. De Boer, S. H., and McCann, M. 1989. Determination of population densities of *Corynebacterium sepedonicum* in potato stems during the growing season. Phytopathology 79:946-951.
14. Douchez, P., Jarnac, J., and Meliot, D. 1982. Le livret du planteur no. 1. La plantation et l'entretien des cannes. Centre Technique de la Canne et du Sucre, Pointe à Pitre, Guadeloupe.
15. Egan, B. T., and Sturgess, O. W. 1980. Commercial control of leaf scald disease by thermotherapy and a clean seed programme. Proc. Int. Soc.

Fig. 11. Relationship between mean disease severity and mean population densities (log base-10 transformation) of *Xanthomonas albilineans* in three parts of 15 clones of the *Saccharum* complex with leaf scald disease (greenhouse experiment 3, 3 months after inoculation).

- Sugar Cane Technol. 17:1602-1606.
16. Feldmann, P., Sapotille, J., Gredoire, P., and Rott, P. 1994. Micropropagation of sugarcane. Pages 15-17 in: *In Vitro Culture of Tropical Plants*. CIRAD-GERDAT (Biotrop), Montpellier, France.
17. Fry, S. M., and Milholland, R. D. 1990. Response of resistant, tolerant, and susceptible grapevine tissues to invasion by the Pierce's disease bacterium, *Xylella fastidiosa*. *Phytopathology* 80:66-69.
18. Harrison, N. A., and Davis, M. J. 1988. Colonization of vascular tissues by *Clavibacter xyli* subsp. *xyli* in stalks of sugarcane cultivars differing in susceptibility to ratoon stunting disease. *Phytopathology* 78:722-727.
19. Hayward, A. C. 1974. Latent infection by bacteria. *Annu. Rev. Phytopathol.* 12:87-93.
20. Jalenques, F. 1988. Dénombrement rapide de colonies microbiennes par le "Système Spiral." *Inf. Technol. Biol.* 1:13-16.
21. Koike, H. 1965. The aluminum cap method for testing sugarcane varieties against leaf scald disease. *Phytopathology* 55:317-319.
22. Koike, H. 1972. Testing sugarcane varieties for leaf scald disease resistance. *Proc. Int. Soc. Sugar Cane Technol.* 14:909-919.
23. Léoville, F., and Coléno, A. 1976. Détection de *Xanthomonas albilineans* (Ashby) Dowson, agent de l'échaudure de la canne à sucre, dans les boutures contaminées. *Ann. Phytopathol.* 8:233-236.
24. Lopes, S. A., Damann, K. E., Hoy, J. W., and Grisham, M. P. 1995. Infectivity titration for assessing resistance to leaf scald among sugarcane cultivars. (Abstr.) *Phytopathology* 85:1121.
25. Martin, J. P., and Robinson, P. E. 1961. Leaf scald. Pages 79-107 in: *Sugarcane Diseases of the World*. Vol. 1. J. P. Martin, E. V. Abbott, and C. G. Hughes, ed. Elsevier Publishing Company, Amsterdam.
26. Mauritius Sugar Industry Research Institute. 1990. *Sugarcane Varieties in Mauritius: A Botanical Description*. Mauritius Sugar Industry Research Institute, Réduit, Mauritius.
27. Miller, J. D., Davis, M. J., Dean, J. L., and Shine, J. M., Jr. 1995. Heritability of resistance to ratoon stunting disease in sugarcane. *Sugar Cane* 1:3-8.
28. Paulet, F., and Glaszmann, J. C. 1994. Biotechnological support for varietal extension of sugarcane. *Agric. Dévelop. (Spec. Issue)* December:47-53.
29. Peros, J.-P., and Lombard, H. 1990. Evaluation rapide de la résistance de la canne à sucre à l'échaudure des feuilles. *Agron. Trop.* 45:139-144.
30. Prior, P., Bart, S., Leclercq, S., Darrasse, A., and Anais, G. 1996. Resistance to bacterial wilt in tomato as discerned by spread of *Pseudomonas (Burkholderia) solanacearum* in the stem tissues. *Plant Pathol.* 45:720-726.
31. Ricaud, C., and Ryan, C. C. 1989. Leaf scald. Pages 39-58 in: *Diseases of Sugarcane: Major Diseases*. C. Ricaud, B. T. Egan, A. G. Gillaspie Jr., and C. G. Hughes, eds. Elsevier Science Publishers, Amsterdam.
32. Roach, B. T. 1992. Susceptibility to ratoon stunting disease in the *Saccharum* complex and feasibility of breeding for resistance. *Sugar Cane* 3:1-11.
33. Roach, B. T., and Jackson, P. A. 1992. Screening sugar cane clones for resistance to ratoon stunting disease. *Sugar Cane* 2:2-12.
34. Rossi Machado, G., Jr. 1994. *Sugarcane Variety Notes: An International Directory*. 6th rev. Cooperativa de produtores de cana, açúcar e álcool do estado de São Paulo Ltda—Copersucar. Piracicaba, São Paulo, Brasil.
35. Rossi Machado, G., Jr., and Burnquist, W. L. 1986. *Sugarcane Variety Notes: An International Directory*. 4th rev. Cooperativa central dos produtores de açúcar e álcool do estado de São Paulo—Copersucar. Piracicaba, São Paulo, Brasil.
36. Rossi Machado, G., Jr., and Walker, D. I. T. 1991. *Sugarcane Variety Notes: An International Directory*. 5th rev. Cooperativa de produtores de cana, açúcar e álcool do estado de São Paulo Ltda—Copersucar. Piracicaba, São Paulo, Brasil.
37. Rott, P., Abel, M., Soupa, D., Feldmann, P., and Letourmy, P. 1994. Population dynamics of *Xanthomonas albilineans* in sugarcane plants as determined with an antibiotic-resistant mutant. *Plant Dis.* 78:241-247.
38. Rott, P., Chatenet, M., and Baudin, P. 1988. L'échaudure des feuilles de canne à sucre provoquée par *Xanthomonas albilineans* (Ashby) Dowson. I. Synthèse bibliographique. *Agron. Trop.* 43:236-243.
39. Rott, P., Chatenet, M., Granier, M., and Baudin, P. 1988. L'échaudure des feuilles de canne à sucre provoquée par *Xanthomonas albilineans* (Ashby) Dowson. II. Diagnostic et spectres d'hôtes de l'agent pathogène en Afrique tropicale. *Agron. Trop.* 43:244-252.
40. Rott, P., Davis, M. J., and Baudin, P. 1994. Serological variability of *Xanthomonas albilineans*, causal agent of sugarcane leaf scald disease. *Plant Pathol.* 43:344-349.
41. Rott, P., and Feldmann, P. 1991. Les maladies de la canne à sucre en Guadeloupe: Situation actuelle et méthodes de lutte mises en place. Pages 90-94 in: *Rencontres Internationales en Langue Française sur la Canne à Sucre*. Actes de la Première Rencontre. AFCAS, Nogent-sur-Marne, France.
42. Rott, P., Soupa, D., Brunet, Y., Feldmann, P., and Letourmy, P. 1995. Leaf scald (*Xanthomonas albilineans*) incidence and its effect on yield in seven sugarcane cultivars in Guadeloupe. *Plant Pathol.* 44:1075-1084.
43. Ryan, C. C., Hogarth, D. M., and Croft, B. J. 1980. Leaf scald ratings of foreign and commercial varieties in Queensland. *Sugarcane Pathol. Newsl.* 24:16-18.
44. Somodi, G. C., Jones, J. B., and Scott, J. W. 1989. Relationship of lesion development on resistant and susceptible tomatoes to internal populations of *Xanthomonas campestris* pv. *vesicatoria*. Pages 208-212 in: *Tomato and Pepper Production in the Tropics*. Proc. Int. Symp. Integr. Manage. Practices. S. K. Green, T. D. Griggs, and B. T. McLean, eds. AVRDC Publication 89-317, Shanhu, Taiwan.
45. Somodi, G. C., Jones, J. B., and Scott, J. W. 1991. Populations of *Xanthomonas campestris* pv. *vesicatoria* in lesions of susceptible and resistant tomato genotypes. *Plant Dis.* 75:357-360.
46. Spence, J. A. 1957. The incidence of leaf scald disease of sugarcane in the West Indies. *Proc. Meet. Br. W. Indies Sugar Technol.* 57:140-146.
47. Steindl, D. R. L. 1971. The elimination of leaf scald from infected planting material. *Proc. Int. Soc. Sugar Cane Technol.* 14:925-929.
48. Teakle, D. S., Appleton, J. M., and Steindl, D. R. L. 1978. An anatomical basis for resistance of sugarcane to ratoon stunting disease. *Physiol. Plant Pathol.* 12:83-91.
49. Teakle, D. S., Smith, P. M., and Steindl, D. R. L. 1975. Ratoon stunting disease of sugarcane: Possible correlation of resistance with vascular anatomy. *Phytopathology* 65:138-141.
50. Thompson, V. 1982. A rapid method of testing the resistance of sugarcane to leaf scald disease. *Sugarcane Pathol. Newsl.* 29:34-35.
51. Walker, D. I. T. 1987. Breeding for resistance. Pages 455-502 in: *Sugarcane Improvement Through Breeding*. D. J. Heinz, ed. Elsevier Science Publishers, Amsterdam.
52. Waraitch, K. S. 1979. Screening of sugarcane clones for field resistance to leaf scald disease in India. *Sugarcane Pathol. Newsl.* 23:8-10.
53. Wrather, J. A., Sappenfield, W. P., and Baldwin, C. H. 1986. Colonization of cotton buds by *Xanthomonas campestris* pv. *malvacearum*. *Plant Dis.* 70:551-552.